Allergology International. 2005;54:427-433

ORIGINAL ARTICLE

The Phosphodiesterase 4 Inhibitor Prevents Antigen-induced Biphasic Nasal Obstruction in Brown Norway Rats

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ABSTRACT

Background: Nasal obstruction is considered the most serious problem for patients with allergic rhinitis. We have previously established a model of nasal obstruction in guinea pigs. In the present study, we tried to establish an allergic model of nasal obstruction using Brown Norway (BN) rats to evaluate the effects of phosphodiesterase (PDE) 4 inhibitors in this model.

Methods: The volume of the nasal cavity was measured with an acoustic rhinometer. Decrease in the volume of the nasal cavity was taken as nasal obstruction. BN rats were actively sensitized with ovalbumin conjugated with aluminium hydroxide. Intranasal antigen instillation induced biphasic nasal obstruction in sensitized BN rats.

Results: Early and late phase responses (EPR and LPR) were observed peaking at 0.5 and 6 hours after the antigen challenge, respectively. Chlorpheniramine did not inhibit EPR or LPR at 10 mg/kg, although the dose was sufficient for the compound to exert its anti-histamine activity. Prednisolone inhibited both responses at 30 mg/kg. Rolipram and CDP-840, PDE4 inhibitors, inhibited both responses at 100 mg/kg. KF19514, a PDE1/4 dual inhibitor, inhibited EPR at 0.1 mg/kg or more and inhibited LPR at 10 mg/kg .

Conclusions: The present study provides a simple model of allergic biphasic nasal obstruction in BN rats, and also suggests that the PDE4 inhibitor may alleviate nasal obstruction in patients with allergic rhinitis.

KEY WORDS

acoustic rhinometry, allergic rhinitis, BN rat, nasal obstruction, PDE inhibitor

INTRODUCTION

Allergic rhinitis is a very common disease. Approximately 20% of the population in industrialized countries suffers from this disease.[1,2] Allergic rhinitis is characterized by sneezing, rhinorrhea and nasal obstruction. When the specific antigen was applied to the nasal cavity of patients with allergic rhinitis, over 90% showed immediate responses, such as sneezing, rhinorrhea and nasal obstruction. Approximately 50% showed late phase responses, such as severe nasal obstruction.[3] Nasal obstruction is considered the most serious problem for patients with rhinitis, because it is quite resistant to all types of drugs except for steroids and decongestants.[4]

Several methods to evaluate the nasal obstruction have been reported.[5-7] Among them, non-invasive acoustic rhinometry was established, characterized and has been used widely in patients with allergic rhinitis.[8,9] This technique was also applied to animal models, such as guinea-pig models.[10] We have recently reported an accurate measurement of nasal obstruction in guinea pigs[11] and also validated the methods by evaluating various types of drugs.[12-14] Brown Norway (BN) rats have been widely used for allergic asthma models because BN rats easily show Th2-dominant IgE production, eosinophilia and airway hyperresponsiveness.[15] However, there have been no

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Received 4 August 2004. Accepted for publication 14 January 2005.

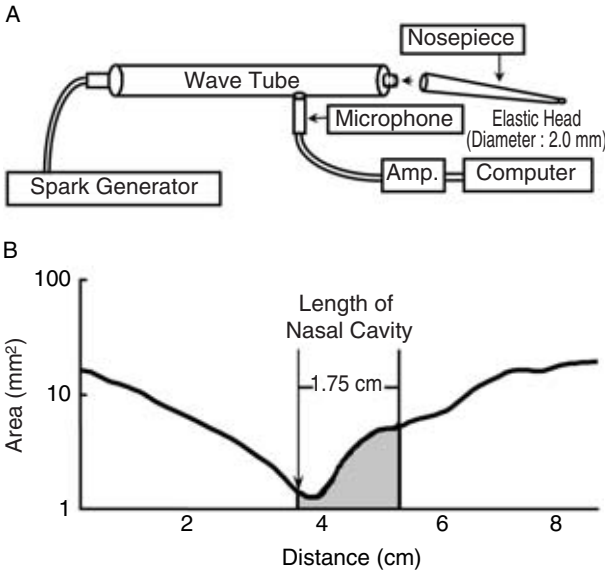

Fig. 1 Schema of the measurement of the volume of the nasal cavity in BN rats. Acoustic rhinometry is a method for the measurement of the volume of the nasal cavity based on the acoustic reflection technique for determining the cross-sectional area as a function of distance in the upper airways. **A**) Diagram of the apparatus in acoustic rhinometry for small animals. **B**) Typical area-distance curve obtained by acoustic rhinometry. The latticed area indicates the volume of the nasal cavity.

reports on nasal obstruction models using rats. It was expected that BN rats would show an obvious nasal obstruction like guinea pigs because of its Th2-dominant immunological properties.

Allergic inflammation should be an important pathogenesis of not only asthma, but also allergic rhinitis.[16] The allergic inflammation can be induced by cytokines such as IL-4, IL-13 and IL-5 produced from Th2 lymphocytes, cationic proteins and prostanoids released from eosinophils, and chemical mediators, cytokines and chemokines released from mast cells. Phosphodiesterase (PDE) 4 inhibitors inhibit such allergic inflammation both in *in vitro* and *in vivo* studies.[17-21] Indeed, the effects of several PDE4 inhibitors have been reported not only in animal models, but also in clinical studies.[22]

KF19514 is a PDE1 and 4 dual inhibitor. Its IC_{50} values in PDE1 and 4 derived from canine smooth muscle are 400 and 270 nmol/L, respectively. KF19514 inhibited antigen-induced bronchoconstriction and airway eosinophilia in guinea pigs. It also inhibited antigen-induced airway hyperresponsiveness at 100 μg/kg, p.o.[23,24]

The present study was done i) to establish a simple model of allergic nasal obstruction in sensitized BN rats, and ii) to evaluate PDE4 inhibitors in this model.

METHODS

ANIMALS

Specific pathogen-free male BN rats (aged 5 weeks) were purchased from Charles River Japan (Yokohama, Japan). All studies were performed in accordance with protocols approved by the Animal Ethical Committee of the Pharmaceutical Research Institute of Kyowa Hakko Kogyo Co., Ltd.

REAGENTS

Chicken egg albumin (OVA; Grade III; Sigma-Aldrich, St. Louis, MO, USA), aluminium hydroxide (Wako Pure Chemicals, Tokyo, Japan) and urethane (Nippon Kasei Chemical, Tokyo, Japan) were used. Chlorpheniramine and prednisolone were purchased from Sigma-Aldrich. Rolipram, CDP-840 and KF19514 were synthesized in our laboratories.

SENSITIZATION AND INTRANASAL CHALLENGE WITH OVA

BN rats were intraperitoneally sensitized with a 1-mL mixture of 1 mg/mL OVA and 100 mg/mL aluminium hydroxide in saline on day 1 and 3. Three weeks after the last sensitization, rats were challenged with the antigen under anesthesia with urethane (1.2 *g*/kg,i.p.). Ten μL of 5% OVA-saline solution was instilled into the bilateral nostrils with a micropipette for antigen-challenge. It was dropped as a bead of fluid on the nostrils, which allowed the rats to aspirate it. Sham-challenged rats received 10 μL of saline in the same manner. All drugs, except for prednisolone, suspended in 0.5% methylcellulose (Wako Pure Chemicals) were given orally in a volume of 10 mL/kg weight 1 hour before the challenge. Prednisolone was given 1.5 hours before the challenge.

EVALUATION OF ANTIGEN-INDUCED NASAL OBSTRUCTION

Decrease in the volume of the nasal cavity was taken as nasal obstruction. The volume of the nasal cavity was measured by acoustic rhinometry. An acoustic rhinometer (GJ Elektronik, Skanderborg, Denmark) was modified by arranging its nosepiece for rats (Fig. 1A). In acoustic rhinometry, a sound pulse created by a spark generator in a wave tube connected with the nasal cavity reflects from the nasal cavity, and the acoustic reflections are measured as a function of the distance from the nostril (Fig. 1B). From this reflection curve, the volume of the nasal cavity from the entrance of the nostril to 1.75 cm depth (the average value from anatomical specimens) was calculated with a computer for each measurement. For each animal, the mean value of 3 measurements for each nostril was regarded as the volume. The nasal obstruction of each animal was evaluated by the sum of the decreases in volume of the left and right nasal cavities. The nasal obstruction was expressed as the per-

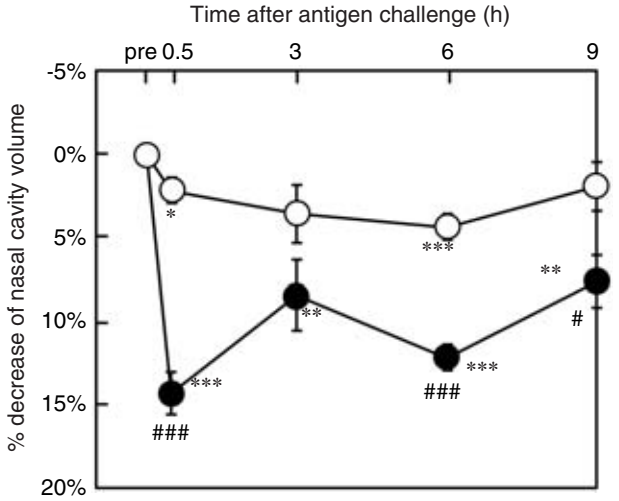

Fig. 2 Time course of the % decrease in the volume of the nasal cavity after saline (open circle) or antigen challenge (closed circle) in actively sensitized BN rats. Saline or antigen solution was instilled 10 μL into the bilateral nostrils of the sensitized rats. The volume of the nasal cavity was measured with an acoustic rhinometer. Data represent the means ± S.E.M. of 8 rats. *, **, *** : $p < 0.05$, 0.01 and 0.001 *vs*. pre value by paired *t*-test, respectively. #, ###: $p < 0.05$ and 0.001 *vs*. the saline group by Student's *t*-test or the Aspin-Welch test, respectively.

centage change from the basal volume of the nasal cavity. The basal volume of the nasal cavity was measured under anesthesia before the antigen challenge.

STATISTICAL ANALYSIS

All data are expressed as means ± S.E.M. Student's *t*-test or the Aspin-Welch test was used for the analysis of the difference between saline-instilled and antigen-challenged groups. Dunnett's or Steel's multiple range test was used for the analysis of the drug effectiveness. A probability value less than 5% was considered as statistically significant. ID_{50} values (dose yielding 50% inhibition) were estimated by Probit method (logistic model).

RESULTS

CHANGES IN THE VOLUME OF THE NASAL CAVITY AFTER THE ANTIGEN CHALLENGE

The volume of the nasal cavity was measured just before and 0.5, 3, 6 and 9 hours after the antigen challenge. The basal volume in the saline-instilled group (30.7 ± 0.2 μL) was not different from that in the antigen-challenged group (30.4 ± 0.2 μL).

The volume of the nasal cavity in sensitized and challenged rats were significantly decreased with a biphasic change peaking at 30 minutes (early phase response; EPR) and 6 hours (late phase response; LPR) after the antigen challenge (Fig. 2), since the

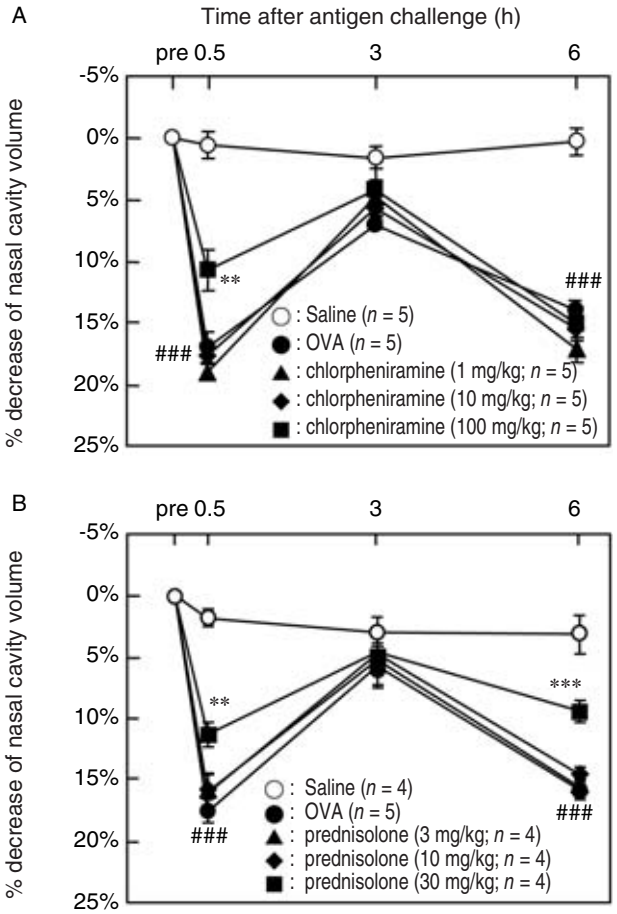

Fig. 3 Effects of chlorpheniramine (**A**) and prednisolone (**B**) on the antigen-induced nasal obstruction in actively sensitized BN rats. Ten μL of saline or antigen solution was instilled into the bilateral nostrils of the sensitized rats. Chlorpheniramine or prednisolone was given orally 1 hour or 1.5 hours before the challenge, respectively. The volume of the nasal cavity was measured with an acoustic rhinometer. Data represent the means ± S.E.M. of 4–5 rats. ###: $p < 0.001$ *vs*. the saline group by Student's *t*-test or the Aspin-Welch test. **, *** : $p < 0.05$, 0.01 and 0.001 *vs*. the challenge group by the Dunnett or the Steel test, respectively.

percentage decreases of the volume of the nasal cavity 30 minutes (14.3 ± 1.3% decrease from the baseline), 6 hours (12.2 ± 0.8% decrease) and 9 hours (7.6 ± 1.6% decrease) after the antigen challenge were significantly greater than in saline-instilled rats. The volume of the nasal cavity 3 hours after the antigen challenge did not decrease.

EFFECTS OF A HISTAMINE H1 ANTAGONIST AND A GLUCOCORTICOID

Chlorpheniramine had little effect on the nasal obstruction in both EPR and LPR at 10 mg/kg, the

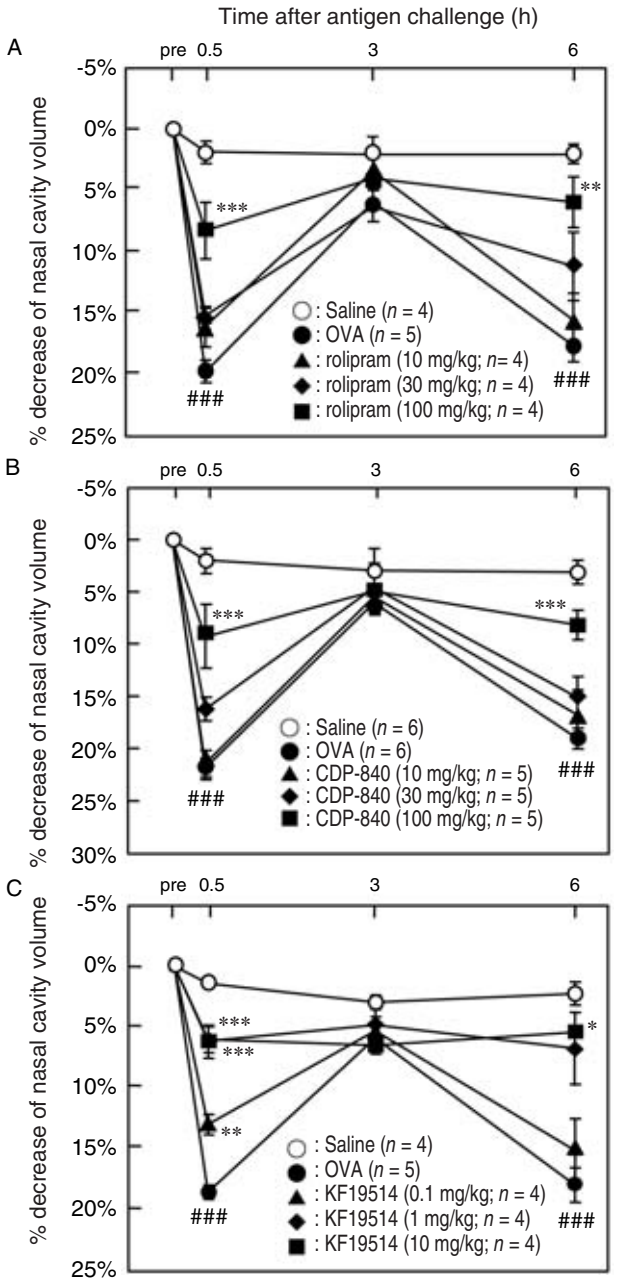

Fig. 4 Effects of rolipram (**A**) , CDP-840 (**B**) and KF19514 (**C**) on the antigen-induced nasal obstruction in actively sensitized BN rats. Ten μL of saline or antigen solution was instilled into the bilateral nostrils of the sensitized rats. Rolipram, CDP-840 or KF19514 was orally given 1 hour before the challenge. The volume of the nasal cavity was measured with an acoustic rhinometer. Data represent the means ± S.E.M. of 4–6 rats. ###: $p < 0.001$ *vs*. the saline group by Student's *t*-test or the Aspin-Welch test. *, **, *** : $p < 0.01$ and 0.001 *vs*. the challenge group by the Dunnett or the Steel test, respectively.

doses sufficient for its histamine H1 antagonistic activity (Fig. 3A) . Prednisolone significantly inhibited both EPR and LPR. The inhibitory effect of prednisolone on LPR (50.4% at 30 mg/kg) was more potent than that on EPR (39.5% at 30 mg/kg) (Fig. 3B). The ID_{50} value of prednisolone in LPR was 30.4 mg/kg (Table 1).

EFFECTS OF PDE4 INHIBITORS

Rolipram (Fig. 4A) and CDP-840 (Fig. 4B) at 100 mg/kg significantly inhibited both EPR and LPR. KF19514 markedly inhibited both EPR and LPR more than 70% at 1 and 10 mg/kg (Fig. 4C). The ID_{50} values of rolipram, CDP-840 and KF19514 in EPR were 62.7, 64.7 and 0.34 mg/kg, respectively. Those of rolipram, CDP-840 and KF19514 in LPR were 41.2, 59.4 and 0.59 mg/kg, respectively (Table 1).

DISCUSSION

The present study provided a simple model of allergic biphasic nasal obstruction in BN rats. The specific antigen induced EPR and LPR, mimicking the early and late phase nasal obstructions in patients with allergic rhinitis.[25] It was suggested that this model depends on inflammation, characterized by the evaluation with a histamine H1 antagonist and a steroid. This model made it possible to evaluate anti-inflammatory drugs for allergic rhinitis easily by using rats. Several PDE4 inhibitors were evaluated in this model.

We previously reported an experimental allergic rhinitis model using guinea pigs which demonstrated a sneezing, nasal rubbing, nasal secretion and nasal obstruction.[12,13] Nasal obstruction was evaluated by measuring the decrease in the volume of the nasal cavity using acoustic rhinometry. Guinea pigs are not sensitive to glucocorticoids because of their high internal glucocorticoids.[26] On the other hand, BN rats are suitable to investigate the mechanism of the allergic inflammation because of their high Th2-dominant immunological profiles.[27,28] BN rats have been used to evaluate various types of drugs for asthma including steroids, because the rats are sensitive to glucocorticoids and produce a biphasic bronchoconstriction by the specific antigen provocation.[29] In addition, rat models are becoming more useful as many immunological reagents including recombinant cytokines and monoclonal antibodies have become available.

To adapt the acoustic rhinometry system to rats, the nosepiece used for guinea pigs was modified to fit rat nostrils. The nosepiece for rats has an elastic head (diameter of 2.0 mm) to perfectly fit the nostrils. In addition, the body posture is critical for measuring the volume of the nasal cavity in rats as well as in humans.[30] It is recommended to measure the volume of the nasal cavity of rats lying on their backs for good reproducibility, though it is still unclear that the volume in this position represents the physiological volume. The reproducibility of the measurement of the volume of the nasal cavity was confirmed by blind tests (data not shown).

Table 1 The values of inhibition % and ID_{50} in early and late phase nasal obstructions

	(mg/kg, p.o.)	Inhibition (% of control)		ID_{50} (mg/kg)	
		0.5 h (EPR)	6 h (LPR)	EPR	LPR
chlorpheniramine	1	<0%	<0%	>100	—
	10	<0%	<0%		
	100	38.3% **	<0%		
prednisolone	3	10.3%	2.2%	>30	30.4
	10	9.6%	9.3%		
	30	39.5% **	50.4% ***		
rolipram	10	20.8%	13.3%	62.7	41.2
	30	25.8%	41.2%		
	100	64.2% ***	75.1% **		
CDP-840	10	2.5%	14.4%	64.7	59.4
	30	28.6%	24.5%		
	100	63.7% ***	67.9% ***		
KF19514	0.1	32.4% **	17.8%	0.3	0.6
	1	72.1% ***	71.3%		
	10	73.3% ***	79.7% *		

Each data represents the mean ± S.E.M. of 4–6 rats. *, **, *** : $p < 0.01$, 0.05 and 0.001 *vs*. the challenge group by the Dunnett or the Steel test, respectively.

In animal models, the nasal secretion and nasal obstruction appear at the same time. In fact, we have seen these symptoms in our experiments with guinea pigs. In guinea-pig models, we measured the nasal cavity volume after the removal of nasal secretion by aspiration. On the other hand, in sensitized BN rats, nasal secretion was not elicited as much, though the nasal obstruction was clearly induced by the antigen challenge. In contrast with guinea pigs, sneezing and nasal rubbing were not observed at all in sensitized conscious BN rats by antigen instillation which is sufficient to induce the nasal obstruction. Though nasal secretion was observed only rarely in BN rats, such rats were easily selected by acoustic wave pattern. In such a case, we removed their nasal secretion by aspiration before measuring nasal volume.

Intranasal antigen instillation induced a biphasic nasal obstruction in sensitized BN rats peaking at 30 minutes and 6 hours after the challenge. This obstruction corresponds to the biphasic one in patients, developing not only at 10–60 minutes but also at 5–10 hours after the intranasal antigen challenge[25] and producing approximately 15–20% of decrease in the volume of the nasal cavity. Chlorpheniramine, a classical H1 antagonist, did not inhibit either response at doses sufficient for showing its anti-histaminic effects. The result that prednisolone inhibited both responses suggests the contribution of inflammatory mechanisms to the development of the nasal obstruction. These results are in agreement with the clinical observations that the nasal obstruction in patients with allergic rhinitis cannot be well controlled by antihistamines, but by steroids.[31-33] In addition, inflammatory mechanisms play a role in the development of the nasal obstruction mainly on LPR, since prednisolone shows a more potent inhibitory effect on LPR by 50.4% inhibition than on EPR by 39.5% inhibition at 30 mg/kg.

There have been no reports on the efficacy of PDE4 inhibitors in experimental allergic rhinitis models. On the other hand, the beneficial effects of PDE4 inhibitors in allergic asthma have been shown in previous preclinical and clinical studies.[22] PDE4 inhibitors might also be effective in allergic rhinitis, because allergic rhinitis and asthma share several epidemiological and pathophysiological factors. The present study clearly demonstrates that three PDE4 inhibitors are effective in blocking both early and late phase nasal obstructions. In addition, the inhibitory effects of PDE4 inhibitors on both EPR and LPR were more potent than those of steroids. It might also be acceptable that KF19514 inhibited the reactions via PDE1 inhibition. However, it was reported that vinpocetine, a PDE1 inhibitor, did not inhibit allergic reactions.[23] It is therefore expected that PDE1 inhibitory activity of KF19514 contributes little to the inhibitory effect on nasal obstruction.

On the other hand, a series of evidence, high concentrations of prostanoids in the nasal lavage fluid after the antigen challenge in patients with allergic rhinitis[34,35] and induction of nasal obstruction by cysteinyl leukotrienes,[36] thromboxane A_2[37] and prostaglandin D_2,[38] have demonstrated their important role in the development of the nasal obstruction. In addition, we have shown that both cysteinyl leukotriene receptor antagonist and thromboxane A_2 receptor antagonist inhibited antigen-induced nasal obstructions on EPR in sensitized guinea pigs.[39] Fur-

thermore, the late phase nasal response has been recognized as an important clinical phenomenon in patients with chronic allergic rhinitis associated with lasting edema of the nasal membrane mucosa, and nasal mucosal infiltrates consisting mainly of eosinophils.[25] It was reported that a PDE4 inhibitor inhibited allergic eosinophilia and release of prostanoids from mast cells and eosinophils.[17-24] It is therefore considered that the potent inhibitory effects of PDE4 inhibitors against the biphasic nasal obstruction are led by the decrease of prostanoid release induced by anaphylaxis on EPR, and by the prevention of eosinophil infiltration into the nasal mucosa and of prostanoid release from those cells on LPR. Indeed, it was recently reported that a PDE4 inhibitor effectively controlled symptoms of patients with allergic rhinitis.[40]

In conclusion, we have demonstrated a simple rat model of allergic nasal obstruction, which develops early and late phase responses. The obstructions do not depend on histamine, but on inflammation. PDE4 inhibitors clearly prevented the allergic nasal obstruction in both phases in our rat model. PDE4 inhibitors may be useful for treatment of allergic rhinitis.

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